

Application No.: 10/526,108
Attorney Docket No.: 47675-102
First Applicant's Name: Sabine Maier
Application Filing Date: 10 May 2006
Office Action Dated: 30 October 2008
Date of Response: 25 November 2008
Examiner: Amanda Marie Shaw

IN THE CLAIMS:

Applicant, pursuant to 37 C.F.R. § 1.121, submits the following amendments to the claims:

1. (Currently amended) A method for the analysis of breast cell proliferative disorders, comprising determining the genomic methylation status of at least one CpG dinucleotide of at least one sequence selected from the sequence group consisting of SEQ ID NOS:1-73, SEQ ID NO:366, contiguous portions thereof, and sequences complementary thereto.
2. (Previously presented) The method of Claim 1, wherein determining the genomic methylation status of the at least one CpG dinucleotide, comprises: obtaining a biological sample comprising genomic DNA from a subject; and contacting the genomic DNA with at least one reagent, or a series of reagents, which distinguishes between methylated and non-methylated CpG dinucleotides.
3. (Withdrawn) A nucleic acid molecule consisting essentially of a sequence at least 18 contiguous bases in length of a sequence selected from the sequence group consisting of SEQ ID NOS:74-365, and SEQ ID NOS:367-370, and sequences complementary thereto.
4. (Withdrawn) An oligomer, consisting essentially of a sequence of at least 10 contiguous nucleotides in length that hybridises to or is identical to a sequence selected from the group consisting of SEQ ID NOS:1-370.
5. (Withdrawn) The oligomer of Claim 4, wherein the contiguous base sequence includes at least one CpG dinucleotide.
6. (Withdrawn) The oligomer of Claim 5, wherein the cytosine of the CpG dinucleotide is located

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in about the middle third of the oligomer.

7. (Withdrawn) A set of oligomers, comprising at least two oligomers according to any of claims 4 to 6.
8. (Withdrawn) The set of oligomers of Claim 7, comprising oligomers for detecting the methylation state of all CpG dinucleotides within sequences of the sequence group consisting of SEQ ID NOS:1-73, SEQ ID NO: 366, contiguous portions thereof, and sequences complementary thereto.
9. (Withdrawn) The set of oligomers of Claim 7, wherein the set is suitable for use as primer oligonucleotides for the amplification of a sequence selected from the sequence group consisting of SEQ ID NOS:1-370, contiguous portions thereof, and sequences complementary thereto.
10. (Withdrawn) The set of oligomers according to Claim 7~~any one of Claims 7 through 9~~, wherein at least one oligomer is bound to a solid phase.
11. (Withdrawn) A method for determining methylation state or for detecting single nucleotide polymorphisms, comprising using a set of oligonucleotides comprising at least three oligomers according to claim 7~~any of claims 4 through 10~~ in an assay suitable for at least one of detecting cytosine methylation state and single nucleotide polymorphisms (SNPs), within a sequence selected from the group consisting of SEQ ID NOS:1-370, contiguous portions thereof, and sequences complementary thereto.
12. (Withdrawn) A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material and suitable for analysing breast cell proliferative disorders associated

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with the methylation state of [[of]] at least one CpG dinucleotide of a sequence selected from the group consisting of SEQ ID NOS:1-73 and SEQ ID NO:366, contiguous portions thereof, and sequences complementary thereto, comprising coupling a set of oligonucleotides comprising at least two oligomers according to claim 7 ~~at least one nucleic acid or oligomer according to any of the claims 3 through 10~~ to a solid phase.

13. (Withdrawn) An arrangement of different oligomers (array) obtainable according to claim 12.
14. (Withdrawn) The arrangement of Claim 13, wherein the oligomers are at least one of oligonucleotides and PNA-oligomer sequences, wherein the carrier material is a planar solid phase, and wherein the oligomers are arranged thereon in the form of a rectangular or hexagonal lattice.
15. (Withdrawn) The arrangement of Claim 13, wherein the carrier material comprises a material selected from the group consisting of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, ~~or~~ gold, and combinations thereof.
16. (Withdrawn) An oligomer array suitable for analysing breast cell proliferative disorders associated with the methylation state of at least one CpG dinucleotide of a sequence selected from the group consisting of SEQ ID NOS:1-73, SEQ ID NO:366, contiguous portions thereof, and sequences complementary thereto, the array comprising a set of oligonucleotides comprising at least two oligomers according to claim 7 ~~at least one nucleic acid or oligomer according to any one of claims 3 through 10~~.
17. (Currently amended) A method for the analysis of breast cell proliferative disorders, comprising:
- a) obtaining a biological sample comprising genomic DNA;

b) contacting the genomic DNA, or a portion thereof with an agent or combination of agents suitable to convert cytosine bases that are unmethylated at the 5-position to uracil or to another base which is dissimilar to cytosine in terms of hybridisation behaviour, to provide a pretreated DNA;

c) amplifying, using at least one set of primer oligonucleotides and a polymerase, at least one pretreated DNA sequence, or a portion thereof, selected from the sequence group consisting of ~~SEQ ID NOS:74-365~~, SEQ ID NOS:367-370, contiguous portions thereof, and sequences complementary thereto; and

d) determining, based on the amplification, or on analysis of the nucleic acid amplificate, the methylation status of one or more genomic CpG dinucleotides, whereby analysis of breast cell proliferative disorders is, at least in part, afforded.

18. (Currently amended) The method of Claim 17, wherein determining in d) comprises hybridisation of at least one nucleic acid or oligomer according to any one of Claims 3 through 6[[10]].

19. (Currently amended) The method of Claim 17, wherein determining in d) comprises hybridisation of at least one oligonucleotide according to any one of Claims 3 through 6[[10]], and extension of the at least one hybridised oligonucleotide-by at least one nucleotide base.

20. (Previously presented) The method of Claim 17, wherein determining in d) comprises sequencing.

21. (Previously presented) The method of Claim 17, wherein amplifying in c) comprises using methylation-specific primers.

22. (Currently amended). The method of Claim 17, wherein determining in d) comprises

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~~hybridisation of at least one nucleic acid or oligomer according to any one of Claims 3 through 18~~
~~use of a combination of at least two of the methods described in any one of Claims 18 through 21.~~

23. (Previously presented) The method of Claim 17, wherein contacting in c) comprises contacting with at least one agent selected from the group consisting of bisulfite, hydrogen sulfite or disulfite.
24. (Currently amended) A method for the analysis of breast cell proliferative disorders, comprising:
- a) obtaining, from a subject, a biological sample containing genomic DNA;
 - b) isolating the genomic DNA;
 - c) digesting the isolated genomic DNA, or a portion thereof comprising at least one sequence selected from the sequence group consisting of ~~SEQ ID NOS 1-73~~, SEQ ID NO:366, contiguous portions thereof, and sequences complementary thereto, with one or more methylation-sensitive methylation sensitive restriction enzymes; and
 - d) detecting the DNA fragments generated in c), whereby analysis of breast cell proliferative disorders is, at least in part, afforded.
25. (Previously presented) The method of Claim 24, further comprising, prior to d), amplifying the DNA digest.
26. (Previously presented) The method of any one of Claims 17 and 25, wherein more than ten different fragments having a length of about 100 to about 200 base pairs are amplified.
27. (Currently amended) The method of any one of Claims 17 and 25[26], wherein amplification of several DNA segments is carried out in one reaction vessel.

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28. (Previously presented) The method of any one of Claims 17 and 25, wherein amplifying is by means of a heat-resistant DNA polymerase.
29. (Previously presented) The method of any one of Claims 17 and 25, wherein amplifying is by means of a polymerase chain reaction (PCR).
30. (Previously presented) The method of any one of Claims 17 and 25, wherein the amplicates carry detectable labels.
31. (Previously presented) The method of Claim 30, wherein said labels are selected from the group consisting of fluorescence labels, radionuclides, detachable molecule fragments having a typical mass which can be detected in a mass spectrometer, and combinations thereof.
32. (Previously presented) The method of Claim 17, wherein amplicates or fragments of the amplicates are detected in the mass spectrometer.
33. (Previously presented) The method of any one of Claims 31 and 32, wherein produced fragments have a single positive or negative net charge for better detectability in the mass spectrometer.
34. (Previously presented) The method of Claim 30, wherein detection is carried out and visualised by means of at least one of matrix assisted laser desorption/ionisation mass spectrometry (MALDI), and using electron spray mass spectrometry (ESI).
35. (Previously presented) The method of any one of Claims 17 and 24, wherein the genomic DNA is obtained from cells or cellular components which contain DNA, sources of DNA

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comprising, for example, cell lines, histological slides, biopsies, tissue embedded in paraffin and all possible combinations thereof.

36. (Withdrawn) A kit reagent having at least one of bisulfite, disulfite, and hydrogen sulfite, as well as at least one of oligonucleotides, and PNA-oligomers according to any one of the Claims 4 through 6[[10]].
37. (Withdrawn) The kit of claim 36, further comprising standard reagents for performing a methylation assay selected from the group consisting of MS-SNuPE, MSP, MethyLight, HeavyMethyl, nucleic acid sequencing, and combinations thereof.
38. (Canceled)
39. (Canceled)
40. (Withdrawn) The oligomer of claim 4, wherein the oligomer is an oligonucleotide or a peptide nucleic acid (PNA)-oligomer.